

Methods of Increasing the Germination of Koa Haole Seed

by

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EXPLANATION OF THE COVER PICTURE

Eight-day old koa haole seedlings from:

- A.* Untreated seed—12 percent germination
- B.* Sulphuric acid-scarified seed—96 percent germination
- C.* Hot water-scarified seed—75 percent germination
- D.* Mechanically scarified seed—92 percent germination

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INTRODUCTION

THE LOCAL IMPORTANCE as a livestock feed (2, 3) assumed by koa haole (*Leucaena glauca*) has resulted in increased planting of this legume. Unfortunately, these plantings have resulted in poor germination, although the potential germination of the seed is 100 percent. It is the purpose of this circular to present some experimental data in regard to practical methods useful in hastening and increasing the germination of the seed of koa haole. The information presented here is the result of studies involving the use of approximately one-third of a million mature seeds collected locally.

When a green feed crop is planted as a soilage crop, it is highly desirable that the seed be quick in sprouting and the resulting stand uniform in order to obtain maximum yield. This circular is intended to assist all those who are interested in obtaining good germination of koa haole, and it is especially intended to assist those who desire quick, uniform stands when this legume is planted as a soilage crop.¹

It is common knowledge that water is necessary for any seed to germinate. Poor germination in koa haole seed is caused by the presence of a very thick, tough, waxy-layered seedcoat which prevents the water from entering the seed. Such a seed is called a "hard seed." Hard seeds are characteristic of many legumes, such as alfalfa, clover, vetch (1), lespedeza (6), and black locust (5). In order to make a hard seed germinate, the nature of the seedcoat must be altered in some way so as to enable the water to enter the seed.

Methods employed to make the seedcoat permeable are called scarification treatments. When koa haole seed is scarified, the seedcoat is punctured or partly destroyed. The result is that water enters the seed and forces it to swell, and the seed germinates. To be sure, the normal seed would germinate eventually as the elements in the soil slowly scarify the seed, but it would take several years before every seed germinated.

GENERAL EXPERIMENTAL PROCEDURE

Preliminary experiments were conducted in which various treatments known to benefit the germination of other seeds were tried. These treatments included: soaking the seed in various chemical solutions (ethyl alcohol, ether, potassium nitrate, chloroform, etc.); subjecting the dry seed to hot and cold temperatures; using various wetting agents; subjecting the seed to various mechanical abrasive agents; and soaking the seed in hot water, or in sulphuric acid. Of these, only the last three treatments were effective in increasing the germination of koa haole seed. These treatments will be described fully in this circular.

When the germination studies were conducted in the laboratory, the seeds were germinated in an electric seed germinator maintained at a temperature of 86° F. Six replications of 50 seeds each were used in each treatment. It was previously determined that the germination results obtained from six replications of 50 seeds each were just as reliable as those obtained from 14 replications. It was also found that at the germinator temperature the seed germinated equally as well as at room temperature. Hence, unless otherwise specified, all germination studies reported in this circular were conducted in the germinator, and six replications of 50 seeds each were

¹ The AAA allows payment for the seeding of koa haole.

used. Only those seedlings with healthy young roots were counted as having germinated. In addition, the numbers of injured seed and sound but ungerminated seed (recorded as hard seed) were recorded.

MECHANICAL SCARIFICATION

Various mechanical means were employed to increase the germination of koa haole seed. These were: rubbing the seed with sandpaper; cutting away or nicking a small part of the seedcoat with a knife; puncturing the seedcoat with a pin or needle; filing the surface of the seedcoat; and subjecting the seed to the abrasive surface on the inside wall of a revolving container of a mechanical scarifier. Of these, only the last is practical in application. Seeds that are mechanically scarified produce a germination of over 90 percent, a result which is considered excellent.

The data presented in Table 1 reveal the effect of mechanical scarification upon germination.

TABLE 1.

MECHANICAL SCARIFICATION WITH BLACK SAND. TWO PARTS SEED TO ONE PART BLACK SAND (APPROXIMATE VOLUME RATIO).

TREATMENT TIME	AVERAGE GERMINATION AFTER					AVERAGE HARD SEEDS	AVERAGE INJURED SEEDS
	2 days	4 days	7 days	10 days	14 days*		
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
5 minutes	21.3	61.6	70.4	76.1	79.4	19.7	0.9
10 minutes	66.7	80.7	88.0	90.0	91.7	6.7	1.6
20 minutes	77.7	91.0	94.0	95.3	96.6	1.7	1.7
30 minutes	77.0	93.3	95.0	96.3	96.6	1.3	2.1
45 minutes	90.0	97.0	98.0	98.0	98.3	0.0	1.7
60 minutes	88.0	97.7	97.7	97.7	97.7	0.3	2.0
Control	2.0	6.7	10.0	13.0	17.7	82.0	0.3

* Difference of 3.8 percentage points between treatment averages is necessary for significance.

According to Table 1, treating the seed for 20 minutes with black sand produced nearly perfect germination, whereas the untreated seed germinated about 17 percent in 14 days. It is also apparent that injury to the seed resulting from the mechanical treatment was negligible.

The data of Table 1 were obtained from one of many trials in which a homemade soil sample shaking machine² (Figure 1) was used as the seed scarifier. The seed together with black sand is placed in a metal container which is fastened to a flat arm of the machine. The arm is eccentrically connected by a piston to a motor-driven wheel. As the wheel revolves, the arm on which the can is attached is jerked upwards and downwards, resulting in a thorough abrasion of the seedcoats as the seeds are thrown against the inside wall of the container and against the sand.

The machine may be easily and cheaply built. The electric motor of about $\frac{1}{4}$ h.p. is the only costly item. The wheel may be obtained from an old sewing machine. The size of the seed container will vary with the amount of seed to be scarified. The greater the amount of seed, the longer

² The writer is indebted to the Soil Chemistry Division for the use of the soil-shaking machine built from a design by Truog, *et al.* (7).

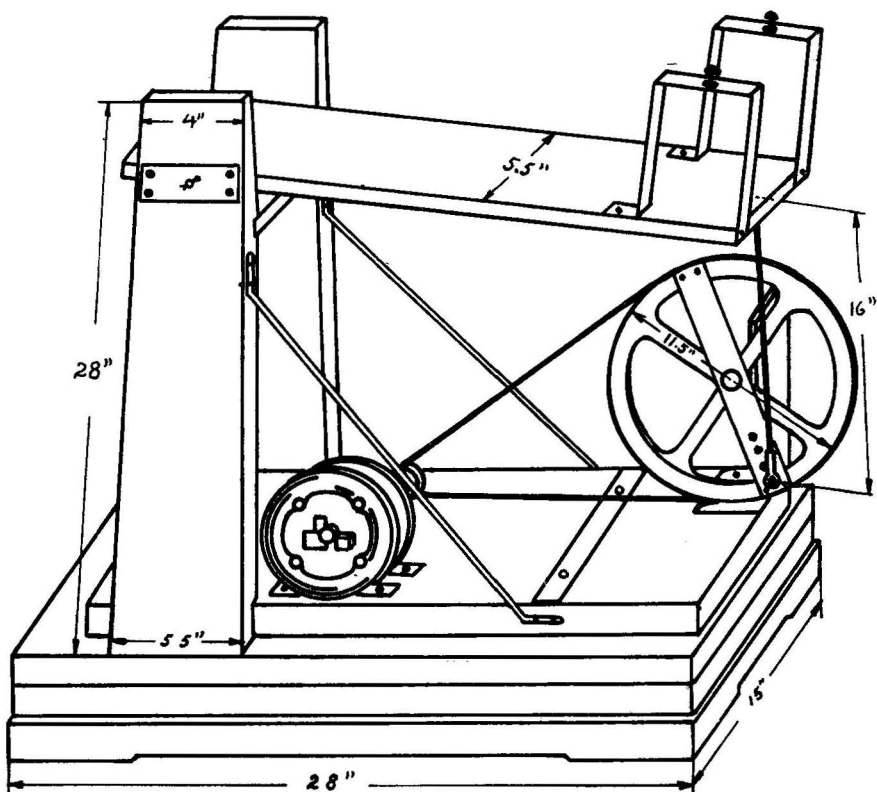


Fig. 1. Mechanical scarifier. Soil sample shaking machine of Truog, *et al.* (7).
(Drawing reproduced with permission.)

will be the time required to scarify. The inner surface of the metal container may be rough or jagged, in which case no sand or gravel is required. The vigorous motion of the machine requires that it be fastened to the floor or to a heavy base. The design of the machine may be altered to meet the user's requirements.

A little experimentation will determine the amount of seed that may be scarified at any one time and the time required to scarify a given seed lot. Simple germination tests may be performed by placing 100 to 200 of the scarified seed in folds of moist cloth or on a piece of moist cloth or paper placed at the bottom of a covered container.

If a concrete mixer is available, it may be used to scarify large lots of seed as suggested by Hurst, *et al.* (4). The mixer bowl, when used as a scarifier, should be kept more nearly horizontal than when mixing concrete. Sand or gravel may be used with the concrete mixer.

Various types of mechanical seed-scarifying machines used for other kinds of leguminous seeds are on the market. These machines can be adjusted for treatment of koa haole seed. Such a machine is owned and

operated by one local meat company³ which treats koa haole seed on a commercial scale.

HOT WATER SCARIFICATION

The possibility of obtaining increased germination of koa haole seed by treating it with hot water was studied. As a result of these studies, the following recommended method to treat this seed with hot water was developed.

EQUIPMENT NEEDED

The equipment needed to treat koa haole seed with hot water includes a metal container, a bag, a fireplace or some other suitable heating device, and a thermometer.

The size of the water container needed will vary with the amount of seed to be treated. Calculations show that $5\frac{1}{2}$ pounds of seed may be treated in a 5-gallon container; 11 pounds in a 10-gallon container; 28 pounds in a 25-gallon container; and 57 pounds in a 50-gallon container. These figures allow for sufficient empty space above the water level to avoid spilling when the seeds are stirred.

DIRECTIONS FOR SCARIFYING SEED WITH HOT WATER

1. Determine the volume of water needed to treat the amount of seed on hand at the rate of one pound of seed to three quarts of water.
2. Add to this volume the volume of water to be displaced by the seed at the rate of one pound of seed to one-third quart of water. The sum is the final volume after the seed is placed in the water.
3. Select a container the volume of which is slightly greater than the final volume.

Example: To determine the volume of water and the size of container required:

Amount of seed to be treated = 40 pounds.

$40 \times 3 = 120$ quarts, volume of water required.

$40 \times \frac{1}{3} = 13$ quarts, volume of water to be displaced by the seed.

$120 + 13 = 133$ quarts (33 gallons), final volume.

\therefore Container required would be one of about 35-gallon capacity or over.

4. Pour the required volume of water into the container and apply heat until the thermometer registers between 167 and 176° F.
5. Remove the heat as soon as this temperature is reached and immediately immerse the bag containing the seed.
6. Manipulate the bag occasionally and let stand until the temperature of the water drops to about 100° F.
7. Remove the bag from the water and spread the seed in a thin layer in a shaded breezy place to dry. Stir occasionally to hasten drying, which is to facilitate sowing.
8. Plant dried seed or hold it for future planting.

The germination results of one of several series of experiments in which koa haole seed was scarified with the above hot water treatment are presented in Table 2.

³ The Hawaii Meat Company in Honolulu scarifies koa haole seed at a nominal fee of a few cents a pound.

TABLE 2.
INITIAL HOT WATER SCARIFICATION.

AMOUNT OF SEED TREATED*	AVERAGE GERMINATION AFTER				AVERAGE HARD SEEDS	AVERAGE INJURED SEEDS	COOLING TIME (to 100° F.)
	4 days	7 days	11 days	14 days†			
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Hour</i>
1 pound	57.7	68.0	77.7	79.3	15.7	5.0	1½
2 pounds	65.0	72.3	77.3	78.3	17.3	4.4	1½
3 pounds	59.4	66.3	75.0	76.3	14.7	9.0	2
4 pounds	57.0	66.3	78.0	80.3	10.7	9.0	2¼
Control	3.4	4.7	5.0	6.0	92.7	1.3	

* Seed was supplied by S. Tachibana of the Horticulture Division.

† Difference of 7.1 percentage points between treatment averages is necessary for significance.

The germination data in Table 2 show that regardless of the quantity of seed treated at any one time, the germination of the treated seed in all cases averaged about the same—more than 70 percent. The treated seeds germinated uniformly. The amount of injury was comparatively low. The time necessary for the hot water to cool increases as the volume of water (and, of course, the amount of seed) increases. In another trial, a six-pound lot was subjected to a similar treatment, and the resulting germination was 75 percent.

Although an initial temperature of between 167 and 176° F. is the recommended temperature of the initial hot water treatment, higher and lower temperatures than this may be used to obtain about the same germination results. However, such temperatures are not suited to practical application. On the one hand, the higher temperatures require so little water that much difficulty is encountered in soaking the seed uniformly, and on the other hand, the lower temperatures require so much water that much difficulty in handling is encountered in the treatment. When one uses the recommended initial of 167 to 176° F., three quarts of water to each pound of seed yields the desired volume ratio of seven to one.

Good germination of koa haole seed may be obtained with the use of hot water of constant temperature, although great care must be used. After many trials, it was found that soaking the seed for 10 minutes in hot water maintained at a constant temperature of 158° F. results in a germination of more than 70 percent. Sustained temperatures above 176° F. are injurious to germination even when the seeds are soaked for very short periods. Temperatures from 104 to 140° F., to be effective, require very long periods of soaking but are never so effective as 158° F. Temperatures below 104° F. are ineffective. From the practical standpoint, constant temperature treatment is of little value because of the difficulty involved in maintaining a constant temperature, especially when large quantities of seed are to be treated.

SULPHURIC ACID SCARIFICATION

PRECAUTIONS AGAINST ACCIDENTS WHEN HANDLING SULPHURIC ACID

1. In mixing acid with water, *always add the acid slowly to the water* in a non-corrosive, heat-resistant container.
2. *Never pour water into the acid.* A violent reaction takes place when water is poured into acid.

3. Do not let any part of the body or clothing come in contact with the acid. Be especially careful about protecting the eyes.

4. If the acid spatters onto skin or clothing, instantly use large quantities of water to wash it off. Very serious burns may result if this precaution is not followed.

Sulphuric acid has been successfully used to scarify hard seeds of other legumes (5). As a result of extensive studies, this acid was found to be very effective with the seed of koa haole also. The following method to scarify this seed with sulphuric acid is recommended.

EQUIPMENT NEEDED

The equipment needed to treat koa haole seed with sulphuric acid is as follows: a 52-percent acid solution prepared from a cheap, crude 78-percent (60° Bé) acid obtainable from a local fertilizer company;⁴ a non-corrosive, heat-resistant container such as glassware or porcelainware or, if a large quantity of acid is to be used, a heavy metal container such as an old oil drum; and a metal sieve with a mesh fine enough to prevent the seed from passing through it.

DIRECTIONS FOR MAKING THE 52-PERCENT ACID

1. Determine the volume of the 52-percent acid needed at the rate of one-third quart of acid to one pound of seed. This ratio allows for a quantity of acid sufficient to cover the seeds completely.

2. Determine the volume of water and the volume of the 78-percent acid needed to make the required 52-percent acid at the rate of one part of water to two parts of acid, that is, one-third of the volume of the 52-percent acid should be made up with water and the remaining two-thirds with the 78-percent acid.

Example: To determine the volume of water and the volume of 78-percent acid required to make the 52-percent acid:

Amount of seed to be treated = 36 pounds.

$36 \times \frac{1}{3} = 12$ quarts, volume of 52-percent acid required.

$\frac{1}{3}$ of 12 = 4 quarts (1 gallon), volume of water required.

$\frac{2}{3}$ of 12 = 8 quarts (2 gallons), volume of 78-percent acid required.

3. Pour the required volume of water into a non-corrosive, heat-resistant container.

4. *Very slowly* pour the required volume of acid into the water, stirring constantly.

5. Cool the resulting hot 52-percent acid solution by floating the acid container in cold water or by allowing cold water to run down its side. The cooled acid may be used immediately, but if it is to be kept for future use, it must be stored in a stoppered glass container.

DIRECTIONS FOR SCARIFYING KOA HAOLE SEED WITH ACID

1. Put the seed into the prepared 52-percent acid.

2. Allow the seed to soak for an hour at room temperature. Stir occasionally.

⁴ The Pacific Guano & Fertilizer Company in Honolulu.

3. After the treatment, drain the acid through a metal sieve, and recover the drained-off acid.

4. Wash the treated seed thoroughly with fast running water. The initial flush of water on the seed must be in a large volume to prevent undue heating.

5. Lay the washed seed in a thin layer in a shaded breezy place to dry. The drying is to facilitate sowing, as in the case of the hot water-treated seed. The dried seed may be planted immediately or kept for future planting.

6. If the recovered acid is to be used again, add to it the 78-percent acid at the rate of one-fourth of the volume of the recovered acid and let it cool before using. (If the volume of the recovered acid is one gallon, add one quart of the 78-percent acid to it.) Thus by this method, the same acid may be used over and over.

The germination results of one experiment in which the koa haole seed was treated with sulphuric acid according to the above directions for recovered acid are recorded in Table 3.

TABLE 3.
SCARIFICATION WITH 52-PERCENT SULPHURIC ACID.

TREATMENT	AVERAGE GERMINATION AFTER			AVERAGE HARD SEEDS	AVERAGE INJURED SEEDS
	3 days	5 days	7 days†		
Original 52-percent acid.....	Percent 90.7	Percent 98.0	Percent 98.3	Percent 0.7	Percent 1.0
↓ Recovered + $\frac{1}{4}$ * 78-percent acid	95.3	98.6	98.7	0.0	1.3
↓ Recovered + $\frac{1}{4}$ * 78-percent acid	91.0	97.7	98.0	0.3	1.7
Control.....	5.0	7.3	9.3	90.0	0.7

* Of the volume of the recovered acid.

† Difference of 2.7 percentage points between treatment averages is necessary for significance.

According to Table 3, the addition of some of the 78-percent acid to the recovered acid results in the maintaining of the effectiveness of the original 52-percent acid. The addition of the 78-percent acid at the rate of less than one-fourth of the volume of the recovered acid results in decreased effectiveness of the acid. If the strength of the recovered acid is not maintained, the germination of the treated seed is low. However, if a very large volume of acid in proportion to the volume of seed is used, the recovered acid may be used effectively without the addition of the 78-percent acid.

Sulphuric acid comes as concentrated (94 to 96 percent), C.P. and crude grades, and as 78 percent (60° Bé) crude. These acids may be used to scarify koa haole seed just as effectively as the 52-percent acid, but the greater danger involved, the higher cost, and the greater accuracy and attention necessary make the use of these acids of higher concentration impractical. Furthermore, the 52-percent acid may be easily prepared from the cheap 78-percent acid as directed above. Acid solutions below 50 percent are ineffective.

GERMINATION IN SOIL

The normal germination of koa haole in the field was determined. An area under a stand of koa haole plants was measured and the number of seedlings growing in this area counted. The apparently healthy but ungerminated seeds in the upper two inches of soil were collected and counted. On the basis of the number of seedlings and the number of ungerminated seeds, the approximate germination percentage was determined. In one area, the natural germination was about nine percent; in another area, about one percent; and in still another, about eight percent.

Some of the recovered seeds were treated with sulphuric acid and sowed in the area from which they were collected. The result was that a good stand of koa haole seedlings developed. What seems to be a thick stand of koa haole plants on the roadside may represent only a very small percentage of the plants that would be growing in the same area had all of the seeds which had fallen on the ground germinated. Nature provides for the perpetuation of this species by restraining germination of its seed and thus extending it over many years.

As a final step in the germination studies of koa haole, seeds which were subjected to the sulphuric acid, hot water, and mechanical scarification treatments were planted in soil in the greenhouse. The results of one of several series are reported in Table 4.

TABLE 4.

GERMINATION OF ACID-SCARIFIED, HOT WATER-SCARIFIED, AND MECHANICALLY-SCARIFIED SEED IN SOIL.

TREATMENT	AVERAGE GERMINATION AFTER			
	1 week	2 weeks	3 weeks	4 weeks†
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
52-percent sulphuric acid scarification.....	83.0	91.7	94.3	94.3
↓				
Recovered + ¼* 78-percent acid	87.3	88.7	89.3	89.7
↓				
Recovered + ¼* 78-percent acid	86.0	89.3	90.7	90.7
Initial hot water scarification.....	65.3	78.3	80.3	80.7
Mechanical scarification	83.7	91.7	94.0	94.0
Control	6.7	13.0	18.3	19.0

* Of the volume of the recovered acid.

† Difference of 6.8 percentage points between treatment averages is necessary for significance.

According to Table 4, it is seen that in two weeks, a great majority of the final germination had occurred in the treated and the untreated seed and that in three weeks, the maximum germination had occurred. These results are very similar to those obtained in the germinator.

LONGEVITY OF SCARIFIED SEED

Longevity or life-span of the scarified seed was studied in order to determine the length of time such seed may be stored before planting. A lot of koa haole seed was divided into three groups. The first group was treated with acid, the second with hot water, and the third was untreated. After treatment, these seeds were dried and stored under ordinary storage conditions. The germination of these seeds over a period of years is presented graphically in Figure 2.

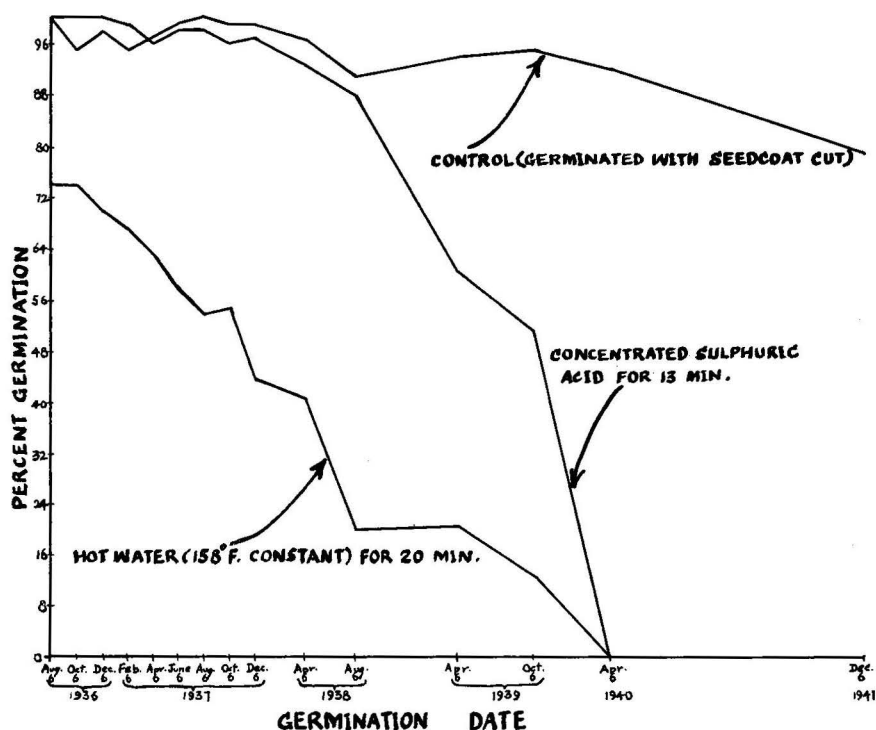


Fig. 2. Longevity of acid-scarified, hot water-scarified, and untreated seed of koa haole in ordinary storage.

According to Figure 2, the acid-scarified seed germinated very well (88 percent) even after two years of storage, but at the end of three years, its germination had dropped to about 50 percent. From then on, the drop in germination was very rapid, and after several months more the seed was dead. The reduction in germination of the hot water-scarified seed was very rapid. This was probably due to the effect of the heat on the embryo. After six months of storage, its germination was 67 percent as compared to its initial germination of 74 percent. At the end of one year, its germination was 54 percent. At the end of two years, its germination was only 20 percent. There was no germination of the hot water-scarified seed at the time the acid-scarified seed was dead.

It must be remembered that some unaffected sound seed is present in the hot water-scarified seed lot, hence the true longevity curves would be slightly (say about 10 percent) above the curve shown in Figure 2. In the acid-scarified seed lot, there is no unaffected seed, hence its curve need not be corrected.

In order to determine the true germination of the normal untreated seed, the seedcoats of these were cut each time the germination test was made. The germination curve of the control (Figure 2) shows that there was little loss of viability in several years; the germination after more than five years of storage was about 80 percent.

In another experiment, some mechanically-scarified seed was stored at room temperature conditions. At the end of three months in storage, the germination of this seed was more than 90 percent. Although the seed was not kept for longer periods, it is expected to keep equally as well as the acid-scarified seed, since the effect of the mechanical treatment and that of the acid treatment is similar.

From the above considerations, it would seem inadvisable to hold in storage the hot water-treated seed of koa haole longer than six months before planting. It is also inadvisable to keep the acid-scarified and the mechanically-scarified seed longer than two years.

EVALUATION OF THE VARIOUS TREATMENTS

From the practical viewpoint the various treatments effective in hastening and increasing the germination of the seed of koa haole may be evaluated.

From the standpoint of initial cost, mechanical scarification is the most expensive. Although the price is variable, factory-made mechanical scarifiers are expensive. However, homemade scarifiers such as the one described in this circular (Figure 1) are rather inexpensive to build. The expense of the acid treatment lies in the cost of the acid, which is comparatively low. There is no expense involved in the hot water treatment.

From the standpoint of thoroughness of scarification, the mechanical treatment and the acid treatment are better than the hot water treatment.

From the standpoint of convenience, mechanical treatment is the easiest method; the hot water treatment, though tedious, is fairly easy and safe; and the acid treatment requires considerable care to avoid injury to the individual as well as containers used. Acid-treated and hot water-treated seed requires drying before sowing, whereas mechanically-treated seed does not require any drying. One can treat more seed per unit time with the mechanical scarifier than with the acid or the hot water treatment.

After taking into consideration these points, it is recommended that if large quantities of seed are to be scarified from time to time, a mechanical scarifier be built or bought and used. It will be a good investment. A home-made scarifier may also be used to scarify small quantities of seed. If small to fairly large quantities of seed are to be treated, either the acid treatment or the hot water treatment should be employed.

When koa haole is planted in rows placed about four feet apart and at the rate of one plant per square foot and about 10,000 seeds to a pound, about five pounds of mechanically-scarified and acid-scarified seed would be required to plant an acre under ideal conditions. Seven pounds of hot water-scarified seed are needed to plant an acre. Under average field conditions, twice these amounts may perhaps be required. By way of contrast, about 60 pounds per acre of the untreated seed may be required to obtain the same number of plants.

SUMMARY

1. Delayed germination in koa haole is due to the impermeable nature of the seedcoat. Scarification treatments alter the nature of the seedcoat in such a way that water enters the seed and makes it germinate.

2. Experimental data have been presented which show the beneficial effect of scarification on the germination of seed of koa haole.

3. Mechanical scarification and acid scarification produce a germination of more than 90 percent. Hot water scarification results in a germination of more than 70 percent. The untreated seed germinates usually about 10 to 15 percent.

4. For practical use, mechanical scarification is recommended for large quantities of seed. For small to fairly large quantities, either the acid or the hot water scarification is recommended.

5. It is inadvisable to hold hot water-treated seed in storage more than six months before planting. Acid-treated and mechanically-treated seed may be safely held for two years.

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